World Academy of Science, Engineering and Technology
International Journal of Environmental and Ecological Engineering
Vol:4, No:10, 2010

Long-term Irrigation with Dairy Factory Wastewater Influences Soil Quality

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***Abstract*—**The effects of irrigation with dairy factory wastewater on soil properties were investigated at two sites that had received irrigation for > 60 years. Two adjoining paired sites that had never received DFE were also sampled as well as another seven fields from a wider area around the factory. In comparison with paired sites that had not received effluent, long-term wastewater irrigation resulted in an increase in pH, EC, extractable P, exchangeable Na and K and ESP. These changes were related to the use of phosphoric acid, NaOH and KOH as cleaning agents in the factory. Soil organic C content was unaffected by DFE irrigation but the size (microbial biomass C and N) and activity (basal respiration) of the soil microbial community were increased. These increases were attributed to regular inputs of soluble C (e.g. lactose) present as milk residues in the wastewater. Principal component analysis (PCA) of the soils data from all 11sites confirmed that the main effects of DFE irrigation were an increase in exchangeable Na, extractable P and microbial biomass C, an accumulation of soluble salts and a liming effect. PCA analysis of soil bacterial community structure, using PCR-DGGE of 16S rDNA fragments, generally separated individual sites from one another but did not group them according to irrigation history. Thus, whilst the size and activity of the soil microbial community were increased, the structure and diversity of the bacterial community remained unaffected.

***Keywords*—**Dairy factory, wastewater; effluent, irrigation, soil quality.

I. Introduction

THE dairy industry is a major source of food processing wastewater [1]. In recent times, rationalization of the dairy industry has seen a trend toward fewer, larger, more automated plants. Large plants can also lead to increased environmental loadings in areas in close proximity to the factory. The dominant environmental concern related to dairy processing is the discharge of large quantities of liquid effluent [1], [2]. The volume and composition of effluents is dependant on the type of product being produced, the nature and scale of the operation and the design of the plant. Factories close to urban areas often subject their effluent to primary and secondary treatment and then discharge it into municipal sewage treatment systems. However, those in rural areas commonly irrigate effluent (after primary and sometimes secondary treatment) onto land surrounding the factory. This is not only a waste disposal strategy for the factory but for farmers it is a supply of irrigation water (often a scarce resource) without the need for reparation. Thus, in the pasture-based, export orientated, dairy industries of New Zealand and Australia, many dairy pastures surrounding factories receive effluent through periodic irrigation. Dairy factory effluent (DFE) generally contains a high organic load, due to the presence of diluted milk/milk products, and also contains significant quantities of cleaning and sanitizing compounds (e.g. NaOH, H_3PO_4/HNO_3, NaOCl).

Environmental concerns surrounding DFW irrigation include the possibilities of leaching of nutrients to groundwater and accumulation of Na in the soil [1]. Nevertheless, a limited amount of research has shown that effluent irrigation has positive effects on soil properties including a liming effect and sometimes modest increases in soil organic matter status [3], [4]. In recent times, much focus has been placed on the concept of maintaining and improving soil quality and soil health [5]. Soil quality can be defined as the capacity of the soil to function, sustain biological productivity, maintain environmental quality and promote plant and animal health [6]. The concept of soil quality considers the soil as a below-ground ecosystem and recognises that soil biological properties and processes are interlinked with chemical and physical ones. Soil microbial activity is, indeed, an important consideration since microbially-mediated processes are central to soil functioning [7]. Important processes include decomposition of organic residues, transformations of soil organic matter, mineralization of nutrients and formation and stabilization of soil aggregates. Key microbial/biochemical measurements include the size, activity, composition and diversity of the soil microbial community. Despite this, surprisingly little research has focussed on the effects of effluent disposal on soil microbial activity. Interestingly, both Degens et al [3] and Sparling et al. [4] observed that DFE irrigation tended to increase the size and activity of the soil microbial community but the wider applicability of such observations is, as yet, unknown.

The purpose of this study was to investigate how DFE irrigation of grazed dairy pastures surrounding a dairy factory influenced soil chemical (pH, EC, organic C, total N, extractable Ca, Mg, K, Na, P) as well as microbial properties including microbial biomass C and N, basal respiration and structure of the bacterial community measured using PCR-

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DGGE of 16S rDNA fragments. For this purpose, two fields with a long-term (> 60 years) history of DFE irrigation and two adjoining fields that had never received DFE were sampled as well as another seven fields from a wider area around the factory.

II. METHOD AND MATERIALS

The experimental site is in the Bega Valley (NSW). Soils in the study area are light textured, alluvial soils and are classified as granitic Chromasols and Tenosols [8]. Experimental monitoring transects were laid down by the Victoria Department of Agriculture in 32 fields surrounding the factory in 2001 [9]. Typical factory effluent content is BOD_5 = 3438, P = 46, N = 161, Ca = 71, Mg = 16, K = 536, Na = 340 mg L^{-1}. For this study, initially two sites close to the factory were chosen that had a known history of long-term (i.e. > 60 years) DFE irrigation [long-term DFE (1) and (2)]. Two fields close-by, which had not received DFE, were chosen as control sites (one irrigated and one dry-land) [control (1) and irrigated control]. The transect lines were split into three equal lengths. Twenty soil samples (0-10 cm) were taken from the three areas (within a distance of 3 m out either side of the line within each of the three lengths). Samples from each area were bulked to give three replicate samples per field. Bulked samples were thoroughly mixed and sieved (< 2 mm). A portion of each sample was stored at 4ºC for microbial analysis and the rest was air-dried and stored for chemical analysis.

A further seven sites were chosen to provide an overall picture of DFE irrigation in the wider area surrounding the factory. These included (i) an additional long-term DFE irrigated site [long-term DFE(3)], (ii) three short-term DFE-irrigated sites [short-term DFE (1), (2) and (3)], (iii) two additional non-irrigated control sites [control (2) and (3)] and (iv) a site under undisturbed native bushland vegetation [native vegetation]. Other than the native vegetation site, all fields were under pasture grazed with dairy cows.

Electrical conductivity and pH were analysed in a 1:5 (v/v) water extract using glass electrodes. Exchangeable bases were extracted with 1 M ammonium acetate (pH 7) and Ca, Mg, K, and Na in the extracts were analysed by ICP-AES [10]. Available P was extracted with 0.5 M $NaHCO_3$ (pH 8.5) (1:100 w/v for 16 h) [10] and measured colorimetrically by the molybdenum blue method. Organic C and total N content were measured by automated dry combustion using a Carlo Erba C, H, N analyser.

Microbial biomass C and N were estimated based on the difference between organic C and N extracted with 0.5 M K_2SO_4 from chloroform-fumigated and unfumigated soil samples using a K_C factor of 0.38 and a K_N factor of 0.54. Basal respiration was determined by placing 30 g oven dry equivalent of moist soil in a 50-ml beaker and incubating the sample in the dark for 10 days at 25ºC in a 1-l air-tight jar along with 10 ml 1M NaOH. The CO_2 evolved was determined by titration. The metabolic quotient was calculated as basal respiration (µg CO_2-C h^{-1}) expressed per mg of microbial biomass C.

DNA was extracted from soil using PowerSoil DNA kit (Mo Bio laboratory) following manufacturer's instructions. Bacterial 16S rDNA was amplified with universal primer pair 341fGC and 534r. Each reaction mixture contained 17.6 µl of purified water; 2.5µl of 10 X PCR buffer and 2.5µl of 25mM $MgCl_2$; 0.5 µl of 10mM dNTPs, 0.5µl of 20 µM primers (341fGC and 534r) [11], 0.5 µl of each sample DNA; and 0.4 µl of Taq polymerase (Amplitaq Gold®) making up a final volume of 25µl. Eight tubes were set up for each sample. PCR products for each sample were combined and purified using a QIAquick PCR purification kit (Qiagen) as recommended by the manufacturer's instructions. PCR cycle used a touch down protocol with an annealing temperature reduction from 68-55ºC (1º per cycle) and an annealing temperature of 55ºC for another 40 cycles. Each cycle consisted of a denaturation at 94ºC for 1 min., an annealing temperature for 1min. and an extension temperature of 72ºC for 1 min. The PCR products were analysed using 2% agarose gel stained with ethidium bromide and visualized under UV. The product was stored in -20°C. DGGE was performed with 8% acrylamide gel containing a chemical gradient from 40% to 70% (7M urea and 40% v/v formamide). 15µl of PCR products were loaded to each lane and electrophoresed in 1X TAE buffer at a constant voltage of 55V for 18 hours. The gel was then stained with ethidium bromide for visualization under UV. DGGE band position and intensity and gel comparisons were assessed using Phoretix ID Pro imaging software (Totallab, Newcastle upon Tyne) and each band position was checked manually and adjusted if necessary.

The data from soil analysis of the 11 sites was subjected to principal component analysis (PCA) using the Minitab Statistical Software Package. Similarly, DGGE band intensity data was subjected to PCA analysis using Minitab.

III. RESULT AND DISCUSSION

Long-term irrigation of effluent with a high Na content will, as shown in Table 1, inevitably result in accumulation of exchangeable Na in the soil. Although, in general, monovalent cations are held less strongly on cation exchange sites than divalent ones, by mass action the added Na displaces other cations (e.g. Ca and Mg) into soil solution and they can then be leached down the soil profile. A decrease in exchangeable Ca and Mg is therefore commonly reported where Na-enriched effluents have been repeatedly applied. At these sites, a decrease in exchangeable Mg was evident (Table 1) but exchangeable Ca levels were not greatly changed. This is presumably attributable to the application of about 3 t ha^{-1} of gypsum ($CaSO_4.2H_20$) in early 2007 which was applied to counteract previous accumulation of exchangeable Na that had occurred at the long-term irrigated sites [9]. The elevated exchangeable Na noted here will be the result of residual Na remaining after the gypsum application plus that which has

TABLE I
SOME CHEMICAL PROPERTIES OF SOILS UNDER LONG-TERM IRRIGATION WITH DAIRY FACTORY EFFLUENT (DFE) COMPARED TO CONTROL SITES

Treatment	pH	EC	Extractable P	Exchangeable Cations				
				Ca	Mg	K	Na	ESP
	($CaCl_2$)	(d S m^{-1})	mg P/ g soil	mmolc kg^{-1}				(%)
Control (1)	4.2a	0.04a	61.8b	58a	22b	2.7a	2.7a	1.7a
Irrigated control	5.5b	0.06a	7.8a	54a	23b	2.3a	1.3a	3.2a
Long-term DFE (1)	6.5c	0.31c	629d	64a	13a	11.2b	11.5b	11.2b
Long-term DFE (2)	6.3c	0.20b	439c	95b	16ab	13.9b	12.2b	8.9b

Means followed by the same letter are not significantly different $P<0.05$.

accumulated since the application. Effluent irrigation also, as expected, increased soluble salt levels and, because of its significant K content, exchangeable K levels were also elevated (Table 1). The increase in pH is attributable to the high pH of DFE (7-8). This increase in pH will need to be monitored since values are 6.3-6.5 in 0.01 M $CaCl_2$ (about 7 in water) and further increases could induce micronutrient cation deficiencies (e.g. Fe, Mn, Zn and Cu).

Although EC values were moderate in long-term DFE-irrigated irrigated soils, ESP values of 9-11% reflect sodic conditions [12]. Nonetheless, dispersive behaviour has not been noted in these soils in the field and subsequent aggregate stability measurements (by wet sieving) have revealed stable aggregation under both control and DFE irrigation. The reason for the stable structure is probably related to the fact that these soils are under permanent pasture. Under pasture, the extremely ramified root system of grasses explores a large proportion of the surface soil and carbohydrate exudates from the roots themselves, and from the extensive rhizosphere microflora have an aggregating and stabilizing effect on soil aggregates [13]. In addition, organic materials in the DFE (e.g. lactose) may also have a stabilizing effect [14].

Extractable P levels are very high under long-term irrigated sites (i.e. 439 and 629 mg kg^{-1}) (Table 1) reflecting the high P content of dairy factory effluent (due principally to the use of H_3PO_4 as a cleaning agent). Degens et al. [3] also noted a large accumulation of extractable and total P in soils under long-term DFE irrigation. Accumulation of P in the surface soil could result in increased losses of P via runoff. Nonetheless, under permanent pasture, where the surface soil is protected by vegetation, such losses are likely to be small. Due to strong adsorption onto inorganic soil colloids, it is usually considered there is a low risk of P leaching down the soil profile. Some studies have, however, suggested that at high soil test P levels measureable movement of P down the profile can occur [15]. It will, therefore, be important to monitor for any possible downward movement of P in these long-term DFE-irrigated soils.

Long-term DFE irrigation did not result in significant increases in organic C or total N in the soil profile (data not shown). The lack of any increase in organic C following long-term effluent irrigation suggests that the additional inputs of organic C in effluent are balanced by losses of C of the same magnitude. Similar results were recorded by Degens et al. [3] and Sparling et al. [4] on long-term DFE-irrigated soils in New Zealand. Under any particular management system, soil organic matter characteristically equilibrates to a level where inputs and losses balance [13]. Losses of C are likely to be principally as CO_2 evolution due to the increased microbial activity (Table 2) but leaching of soluble organic matter could also play a part (Menneer et al. [16]. The lack of any accumulation of total N in the soil under DFE irrigation may well be at least partially related to similar N loads being applied to the "control" and DFE-irrigated fields. That is, control sites typically received substantial inorganic fertilizer N inputs (i.e. 100-200 kg ha^{-1} yr^{-1}) while fertilizer rates were much reduced on effluent-irrigated fields (to take account of N inputs from the effluent).

The increase in the size (microbial biomass C and N) and activity (basal respiration) of the soil microbial community under DFE irrigation (Table 2) is likely to be the result of regular inputs of soluble C (e.g. lactose), along with additional N and P, in the effluent. This occurred despite there being no increase in total soil organic C content. Such an increase in microbial biomass, despite no increase or even a decrease in organic C, was also noted by Sparling et al. [4] in long-term DFE irrigated pastures. There was no increase in metabolic quotient due to DFE irrigation (Table 2) indicating that the increase in basal respiration was proportional to the increase in microbial biomass C. An increase in metabolic quotient is considered a response of the microbial community to adverse conditions (either stress or disturbance) [17]. Thus, the accumulation of soluble salts, P and Na in the effluent-treated soil did not appear to cause undue stress to the soil microbial community.

When all the soil parameters measured (pH, EC, organic C, total N, C:N ratio, Soluble C, soluble N, microbial biomass C, basal respiration, metabolic quotient, exchangeable Ca, Mg, K, Na, Al, ESP, ECEC and extractable P) for all 33 samples (at the 11 sites) were subjected to principal component analysis (Fig. 1a) the long-term irrigated sites were separated on the PC1 and PC2 ordinates from the non-DFE irrigated sites and short-term irrigated sites tended to fall between the two former groups. The ordination biplot of loading scores is shown in

TABLE II
Microbial biomass C and N, basal respiration and metabolic quotient in soils under long-term irrigation with dairy factory effluent (DFE) compared to control sites.

Treatment	Microbial biomass C	Microbial biomass N	Basal respiration	Metabolic quotient
	mg kg^{-1}	mg kg^{-1}	µg C g^{-1} day^{-1}	µg C mg^{-1} day^{-1}
Control (1)	164b	30.3a	25.8a	0.16ns
Irrigated control	126a	50.7b	22.3a	0.12
Long-term DFE (1)	261c	70.4c	36.5b	0.14
Long-term DFE (2)	282c	72.8c	34.8b	0.12

Means followed by the same letter are not significantly different P<0.05.

Fig. 1b. This indicates that effluent-irrigated soils are characterized by high EC, ESP, exchangeable Na and K and microbial biomass and a low C/N ratio. This supports the data presented in Table 1 and 2 and the conclusion of Liu and Haynes [18] that the main effects of DFE irrigation on soil chemical properties are an increase in exchangeable Na and ESP, an accumulation of soluble salts, an accumulation of P and a liming effect. In addition, a narrowing of the C/N ratio is a common occurrence in DFE-irrigated soils due to the inputs of effluent N [4], [14].

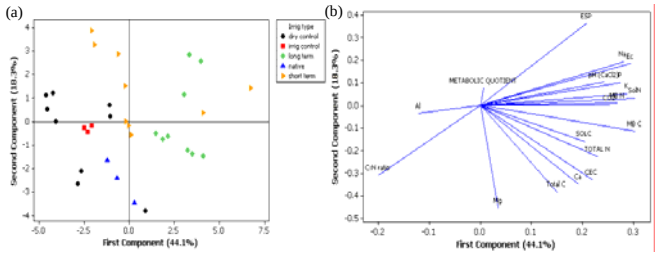

Fig. 1 (a) Ordination biplot of principal component analysis of 19 soil chemical and microbial properties of the 11 experimental sites, (b) Ordination biplot of loading scores for the 19 soil properties

DGGE fingerprinting of bacterial 16S rDNA amplicons from each of the 33 soil samples contained 23-32 bands, with some common to all lanes but varying with respect to relative band intensity. Visual observations showed that banding patterns for replicate samples from each field were highly repetitive, consistently more similar to each other and distinct from those in other fields, indicating high interspecies variation between fields. The ordination biplot for PCA analysis of DGGE banding patterns for soils from the 11 sites is presented in Figure 2. In general the three replicate samples from each site clustered together but there was no grouping of sites according to long-term, short-term or no DFE irrigation. That is, heterogeneity in DGGE banding patterns between soils from different fields with a similar history of DFE irrigation (or control) was such that no change in pattern due to DFE irrigation was observed. Thus, although DFE irrigation increased both the size and activity of the soil microbial community the structure and diversity of the soil bacterial community remained unaffected. From a soil quality/health viewpoint, data reported here are encouraging in that, despite substantial changes in soil chemical properties, the structure of the bacterial community has remained unaffected by long-term DFE irrigation whilst its size and activity has, in fact, increased.

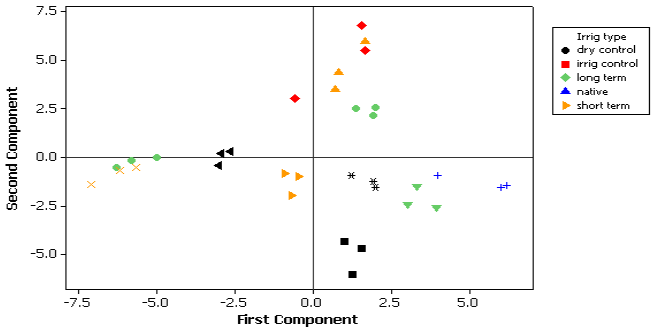

Fig. 2 Ordination biplot of principal component analysis of bacterial DGGE patterns from the 11 experimental sites

IV. Conclusion

Under DFE irrigation there is a characteristic increase in EC, ESP, extractable soil Na, K and particularly P, and an increase in the size and activity of the soil microbial community. Despite these changes, the metabolic quotient and structure of the soil bacterial community remained unaffected suggesting that the existing soil microbial community is relatively resilient under DFE irrigation. Further work will investigate the effects of DFE irrigation on the catabolic diversity of the soil microbial community.

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